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A Protective Chaperone for the Kinetochore Adaptor Bub3

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In this issue of *Developmental Cell*, two complementary studies by Jiang et al. (2014) and Toledo et al. (2014) identify BuGZ as an interacting protein of the kinetochore adaptor Bub3 and show that it promotes the stabilization and kinetochore loading of Bub3, chromosome alignment, and mitotic progression.

High-fidelity chromosome segregation prevents aneuploidy and maintains genome stability. To achieve faithful chromosome segregation, the spindle checkpoint delays anaphase onset in response to kinetochores not properly attached to spindle microtubules (Jia et al., 2013). Several spindle checkpoint proteins, including Bub1, BubR1, and Bub3, also actively promote correct kinetochore-microtubule attachment and chromosome alignment at the metaphase plate. These three proteins form two heterodimers, Bub1-Bub3 and BubR1-Bub3, with Bub3 directly binding to the GLEBS motif in Bub1 or BubR1. Both the spindle-checkpoint and chromosome-alignment functions of Bub1/BubR1-Bub3 require their kinetochore targeting.

Recent studies have shown that, in mitosis, Bub3 binds phosphorylated MELT motifs in the kinetochore scaffolding protein Knl1 and recruits Bub1 and BubR1 to kinetochores (Krenn et al., 2014; Primorac et al., 2013). Reporting in this issue of *Developmental Cell*, two groups now demonstrate the involvement of *Bub*3-interacting *G*LEBS-motif-containing *Z*NF207 protein (BuGZ) in proper chromosome alignment through promoting the kinetochore targeting of Bub1/BubR1-Bub3 (Jiang et al., 2014; Toledo et al., 2014).

In a focused RNA interference (RNAi) screen targeting spindle matrix proteins in mouse embryonic stem cells, Jiang et al. (2014) found that depletion of BuGZ caused prolonged mitotic arrest followed by cell death. Likewise, in an RNAi screen targeting putative transcription factors, Toledo et al. (2014) independently identified BuGZ as specifically required for the viability of glioblastoma stem cells, but not for that of nontransformed neural stem cells. Both groups then identified Bub3 as a BuGZ-binding partner. BuGZ contains an N-terminal zinc finger domain and a conserved GLEBS motif that mediates Bub3 binding. While Bub3 is conserved from yeast to man, BuGZ orthologs exist only in metazoans.

Both groups reported that BuGZ depletion decreased the levels of Bub3 protein in human cells without affecting the levels of Bub3 mRNA (Jiang et al., 2014; Toledo et al., 2014). Moreover, ectopic expression of an RNAi-resistant wild-type BuGZ, but not a mutant with its GLEBS motif mutated, restored Bub3 levels in BuGZ RNAi cells. These results suggested that direct binding of BuGZ stabilizes the Bub3 protein. The mechanism by which BuGZ protects Bub3 remains unclear, as the two studies disagree on the involvement of proteasome in this process. Jiang et al. showed that addition of the proteasome inhibitor MG132 restored Bub3 levels in BuGZ-depleted mitotic *Xenopus* egg extracts. In contrast, Toledo et al. did not observe a restoration of Bub3 levels in BuGZ RNAi cells treated with MG132. It is possible that BuGZ protects Bub3 from both proteasome-dependent and -independent degradation pathways (Figure 1A), and the relative contributions of these pathways differ in different systems.

Depletion of BuGZ diminished, but did not abolish, the kinetochore localization of Bub3, Bub1, and possibly BubR1 (Jiang et al., 2014; Toledo et al., 2014). Consistent with the known roles of Bub1 and BubR1 in chromosome alignment, BuGZ depletion also caused chromosome-alignment defects. These cells then experienced a checkpoint-dependent mitotic arrest followed by cell death, presumably because the residual Bub1 and BubR1 at kinetochores still mounted a robust spindle checkpoint response.

Is the defective kinetochore localization of Bub3 in BuGZ-depleted cells simply the consequence of reduced Bub3 levels? The answer is no. Jiang et al. made a BuGZ mutant with N-terminal zinc-finger region deleted (BuGZΔN). Expression of BuGZΔN in BuGZ RNAi cells restored Bub3 levels but did not restore its kinetochore localization. Thus, in addition to stabilizing Bub3, BuGZ has a more direct role in targeting Bub3 to kinetochores through its N-terminal region. Interestingly, this region of BuGZ binds to microtubules in vitro and targets BuGZ to the mitotic spindle in human cells. These results led Jiang et al. to propose that BuGZ actively loads Bub3 onto kinetochores in a microtubule-dependent manner (Jiang et al., 2014).

Because Bub1, BubR1, and BuGZ all use their GLEBS motifs to interact with Bub3, their binding to Bub3 is presumably mutually exclusive. BuGZ is recruited to kinetochores during prophase and prometaphase by Bub3 and Knl1 (Toledo et al., 2014). The kinetochore-bound BuGZ is thus expected to compete with Bub1/BubR1 for kinetochore targeting. How, then, does BuGZ promote the kinetochore recruitment of Bub1/BubR1? Both groups proposed a relay model to explain this paradox (Figure 1B, model i). In this model, BuGZ initially recruits Bub3 to the kinetochores, possibly with the help of microtubules. Upon docking with phospho-MELT (pMELT) motifs of Knl1 at kinetochores, BuGZ dissociates from

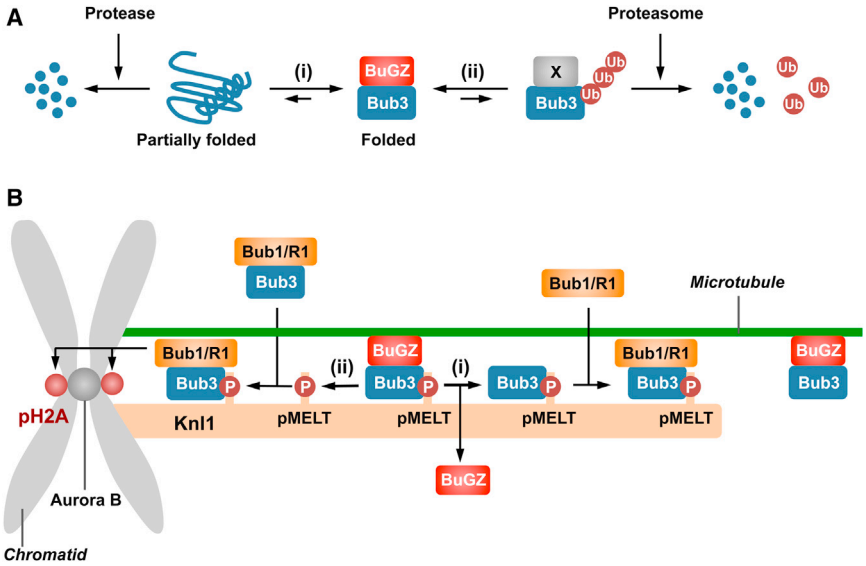

Figure 1. The Kinetochore Adaptor Bub3 Needs Its Protective Chaperone BuGZ to Recruit Bub1 and BubR1 to Kinetochores

(A) Two nonexclusive mechanisms by which BuGZ stabilizes Bub3: (i) BuGZ stabilizes the folded state of Bub3 and guards against its proteolysis; (ii) BuGZ blocks binding of ubiquitination enzymes to Bub3 and prevents its ubiquitination and proteasome-mediated degradation.

(B) Two models for BuGZ-mediated kinetochore targeting of Bub1 and BubR1: the relay model (i) and the priming model (ii). Kinetochore-bound Bub1-Bub3 installs the phospho-H2A mark, which contributes to centromere targeting of Aurora B.

and frees Bub3, allowing it to recruit Bub1/BubR1. Consistent with this model, the kinetochore localization of BuGZ appeared to be transient and disappeared by metaphase (Toledo et al., 2014). In fact, Jiang et al. did not observe prominent kinetochore localization of BuGZ, but instead showed clear BuGZ localization to the mitotic spindle (Jiang et al., 2014).

This model is somewhat at odds with the fact that Bub1/BubR1-Bub3 exist as constitutive, stoichiometric complexes throughout the cell cycle. It is unclear whether there are cytosolic Bub1 and BubR1 molecules not bound to Bub3 that can be recruited by the free Bub3 installed by BuGZ at kinetochores. We envision an alternative priming model (Figure 1B, model ii), which allows BuGZ-facilitated kinetochore recruitment of intact Bub1/BubR1-Bub3 heterodimers. Because Knl1, the kinetochore receptor for Bub3, contains multiple, functional MELT motifs in tandem, binding of BuGZ-Bub3 to one such motif may cause a conformational change in Knl1 and expose neighboring motifs, possibly through the N-terminal zinc-finger domain of BuGZ. This BuGZ-dependent priming of Knl1 could promote the recruitment of Bub1/BubR1-Bub3 heterodimers to kinetochores. Bub1/BubR1 can establish additional Knl1 interactions that synergize with the Bub3-pMELT interaction (Krenn et al., 2014), resulting in stable binding of Bub1/BubR1-Bub3 at kinetochores. Future experiments are needed to test these and other models.

How does kinetochore-bound Bub1 regulate chromosome alignment? The molecular details remain to be established but appear to involve multiple mechanisms that regulate the kinase Aurora B. Aurora B promotes chromosome alignment through phosphorylating a critical microtubule receptor, the Ndc80 complex, and breaking improper microtubule-kinetochore attachments. The centromeric localization of Aurora B requires Bub1-dependent histone H2A phosphorylation at kinetochores (Liu et al., 2013; Yamagishi et al., 2010). Consistent with the reduced kinetochore targeting of Bub1 in BuGZ-deficient cells, phosphorylation of H2A and Ndc80 at kinetochores decreased, indicative of reduced kinase activities of Bub1 and Aurora B (Toledo et al., 2014).

Collectively, these two studies establish BuGZ as a critical regulator of chromosome alignment through mediating the stability and kinetochore loading of Bub3. Many open questions remain. For example, what are the exact functions of the transient pools of BuGZ-Bub3 at kinetochores? Is the microtubule-binding function of BuGZ directly involved in kinetochore-microtubule attachment? Does BuGZ have an active role in checkpoint silencing? Perhaps the most important, unanswered question is why BuGZ is selectively required for chromosome alignment in cancer cells, but not in normal cells. Addressing the molecular basis of this selectivity may reveal new therapeutic targets for cancer therapy.

REFERENCES

Jia, L., Kim, S., and Yu, H. (2013). Trends Biochem. Sci. *38*, 302–311.

Jiang, H., He, X., Wang, S., Jia, J., Wan, Y., Wang, Y., Zeng, R., Yates, J., III, Zhu, X., and Zheng, Y. (2014). Dev. Cell *28*, this issue, 268–281.

Krenn, V., Overlack, K., Primorac, I., van Gerwen, S., and Musacchio, A. (2014). Curr. Biol. *24*, 29–39.

Liu, H., Jia, L., and Yu, H. (2013). Curr. Biol. *23*, 1927–1933.

Primorac, I., Weir, J.R., Chiroli, E., Gross, F., Hoffmann, I., van Gerwen, S., Ciliberto, A., and Musacchio, A. (2013). eLife *2*, e01030.

Toledo, C.M., Herman, J.A., Olsen, J.B., Ding, Y., Corrin, P., Girard, E.J., Olson, J.M., Emili, A., DeLuca, J.G., and Paddison, P.J. (2014). Dev. Cell *28*, this issue, 282–294.

Yamagishi, Y., Honda, T., Tanno, Y., and Watanabe, Y. (2010). Science *330*, 239–243.